S/N 09/508,377
PATENT IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Zhongyi Li, et al	Examiner:	Baum, Stuart F
Serial No:	09/508,377	Group Art Unit:	1638
Filed:	06/09/2000	Docket No.:	054270/0126
Title:	Regulation of Gene Expression in Plants		

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited in the United States Postal Service, as first class mail, in an envelope addressed to:
Assistant Commissioner for patents, Washington, D.C. 20231 on _____

By: _____
Name:

AFFIDAVIT OF DOCTOR SADEQUR RAHMAN

BOX AF

Assistant Commissioner for Patents
Washington D.C. 20231

Dear Sir:

I, Doctor Sadequr Rahman declare and state:

1. That my professional training and experience are documented on the abridged *curriculum vitae* attached as Exhibit 1.

2. That I am currently employed as a Principal Research Scientist by the Commonwealth Scientific and Industrial Research Organisation ("my company"), which is located in Canberra, Australian Capital Territory, Australia. My past employment history is documented on the attached abridged *curriculum vitae.*

3. I am one of the applicants and inventor of the invention covered by United States Patent Application Serial No. 09/508,377.

4. I have read and considered the Examiners Office Action issued on July 2, 2003, the specification of 09/508,377 and the currently pending claims 48-68.

5. In particular I have read the Examiner's comments regarding the utility of the claimed invention. I note that the Examiner contends on pages 4 and 5 of the Office Action that "Given that a closer look at the presented data only indicates that 13 of the 768 amino acids of SEQ ID NO:12 share identity with

the N-terminal region of other starch branching enzymes, and given that it has not been disclosed if the catalytic site of the starch branching enzyme is contained in the N-terminal region of the polypeptide, and given that Applicants isolated their invention by weak sequence hybridisation to a maize starch branching enzyme II, and given that Applicants have not verified the starch branching activity of their polypeptide, Applicant's invention is not supported by either a credible asserted utility or a well established utility."

6. I believe that the specification as filed does indeed provide credible and well established utility for the isolated SBE II-D1 gene of wheat (see SEQ ID NO:10) and the polypeptide encoded thereby. Example 14 (page 37) states "sequencing of the SBE II gene contained in clone 2, termed *SBE II-D1* (see SEQ ID NO: 10), showed that it coded for the N-terminal sequence of the major isoform of SBE II expressed in the wheat endosperm, as identified by Morell *et al.* (1997). This is shown in Figure 13." A copy of Morell *et al.* (1997) is attached as Exhibit 2.

7. I believe that this indicates clearly that the protein encoded by the isolated *SBE II-D1* gene is the same as that identified by Morell *et al.* (1997). Morell *et al.* (1997) identified the protein after purifying it on the basis of its enzymatic activity, having SBE II activity, see pages 202, 203 and Table II on page 205 of Exhibit 2.

8. The second SBE II gene isolated, represented by the clone SBE-9 (see Example 13, page 36 of the specification and encoding the amino acid sequence of SEQ ID NO:12), is a cDNA clone having about 97% sequence identity with the coding region of SEQ ID NO:10 and was subsequently shown to have SBE activity. This was reported in Rahman *et al.* (2001), where the clone is designated cDNA1. A copy of Rahman *et al.* (2001) is attached as Exhibit 3.

9. On page 1316, left-hand column of Rahman et al. (2001) it states:
   "However, cDNA1 encodes a functional branching enzyme as it could complement *Escherichia coli* mutants lacking branching enzyme due to the *glg*B mutation (data not shown)."

10. Page 1321, paragraph spanning the left-hand and right-hand columns, states:
    "It [cDNA1] is very similar to Y11282 [Nair *et al.* sequence] except that it is missing the sequences corresponding to parts of exon 1 and all of exon 2 of *wSBEII-DA1* and consequently does not encode the reported N terminus for SBEII (Morell *et al.*, 1997). Nevertheless this truncated polypeptide is capable of complementing a branching enzyme II mutation in glycogen biosynthesis in *E. coli* (data not shown)."
    These two statements indicate that the SBE-9 cDNA clone described in the specification and encoding the protein having the amino acid sequence of SEQ ID NO:12 has starch branching enzyme activity.

11. The isolated nucleic acid molecule comprising the SBE II-D1 gene (SEQ ID NO:10 was isolated by hybridisation under stringent conditions (Example 1, page 20) to an SBE-9 probe, as described in Example 13, paragraph spanning

pages 36 and 37 of the specification. As stated above, the two nucleotide sequences are about 97% identical in the coding regions. Therefore, the Examiner's point that "Applicants isolated their invention by a weak sequence hybridisation to a maize starch branching enzyme II" (page 5) is of no consequence. The skilled artisan, on reading the specification, would have understood that the SBE II-D1 gene (SEQ ID NO:10) encoded the functional starch branching enzyme described by Morell et al (1997) and the SBE-9 clone (SEQ ID NO:12) were closely related in sequence.

12. Furthermore, the specification states on page 4 lines 22-25, that "the sequences [of Nair et al 1997; Accession No. Y11282, which were reported to encode SBE] are very similar to those reported herein; there are differences near the N-terminal of the protein, which specifies its intracellular location." N-terminal sequences (signal peptides) that are cleaved off proteins such as SBE are known to direct intracellular location or compartmentilization of such proteins. Such signal peptides are not required for enzymatic activity of the mature proteins, only for their localization. Therefore, the Examiner's point "it has not been disclosed if the catalytic site for the starch branching enzyme is contained in the N-terminal region of the polypeptide" (page 4/5) is not relevant and of no consequence.

13. I or others working under my supervision have carried out experiments to down-regulate the expression in hexaploid wheat of the genes corresponding to the *SBE II-D1* gene of *T. tauschii* (SEQ ID NO:10), successfully inactivating SBE and modifying the starch composition of the resultant grain, according to the methods described in the specification. These experiments and the results are described in the following sections.

14. A gene construct for down regulation of SBE II in wheat containing 1536bp of nucleotide sequence from the wheat *SBEII* gene was made as follows. The nucleotide sequence included a 468bp sequence that comprised the whole of exons 1 and 2 and part of exon 3 (fragment 1, nucleotide positions 1058 to 1336, 1664 to 1761 and 2038 to 2219 of SEQ ID NO:10), a 512bp sequence consisting of part of exons 3 and 4 and the whole of intron 3 (fragment 2, nucleotide positions 2220 to 2731 of SEQ ID NO:10) and a 528bp fragment consisting of the complete exons 1, 2 and 3 (fragment 3, nucleotide positions 1058 to 1336, 1664 to 1761 and 2038 to 2279 of SEQ ID NO:10) in reverse orientation. The construct was initially generated in the vector pDVO3000 which contains a HMWG promoter sequence and nos3' terminator, and then the expression cassette inserted into a binary vector that also comprised a sclectable marker gene.

15. The binary vector, containing the inhibitory construct, and appropriate control constructs in binary vectors were used to produce transformed wheat plants of the variety NB1. Transformed plants were regenerated from transformed embryogenic cells that were able to grow in the presence of the selective agent, and shown to be transformed by PCR experiments using primer combinations specific for the introduced gene construct. 31 regenerated plants were obtained from 10 separate transformation experiments and, of these, 27 were shown to be transformed with the inhibitory construct.

16. Grain obtained from the transformed wheat plants was analysed for starch modifications and other phenotypic changes. Of 25 lines analysed, 15 had grain with distorted starch granules, 12 of them had severely distorted granules. There was a significant reduction in birefringence for distorted granules under polarized light. Western blot analysis using specific antibodies showed that levels of SBE II protein were substantially reduced in each of the 4 independently transformed lines examined and were almost undetectable in at least some seeds in each of the lines. Furthermore, the proportion of amylose in the starch as determined by iodometric analysis was considerably enhanced in seeds of 3 out of 4 lines examined and correlated with down-regulation of SBE II gene expression.

17. These experiments confirm that the isolated nucleic acids of the claimed invention including SEQ ID NO:10 may be used according to the teaching of the specification to alter the expression of starch branching enzyme II in a plant and to alter the starch in the plant. They demonstrate that the genes corresponding to SBE II-D1 in wheat encode functional SBE II and confirm the utility of the claimed isolated nucleic acids encoding SBE II such as SBE-9 (SEQ ID NO:12).

18. I declare that all statements made herein on my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful, false statements may jeopardise the validity of the application or patent issuing therefrom.

Respectfully submitted,

By: Sadequr Rahman Date: 31.10.03

Name: Doctor Sadequr Rahman

Title: Principal Research Scientist.